

# Influence of Lactobionic acid on the kinetics of Thrombin in human plasma

Citation for published version (APA):

Beguin, S. S., Dol, F., & Hemker, H. C. (1991). Influence of Lactobionic acid on the kinetics of Thrombin in human plasma. *Seminars in Thrombosis and Hemostasis*, 17(Suppl. 1), 126-128.

## Document status and date:

Published: 01/01/1991

## Document Version:

Publisher's PDF, also known as Version of record

## Please check the document version of this publication:

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# Influence of Lactobionic Acid on the Kinetics of Thrombin in Human Plasma

SUZETTE S. BÉGUIN, Ph.D. FREDERIC DOL, Ph.D., and  
H. COENRAAD HEMKER, M.D., Ph.D.

In clotting plasma, it has been demonstrated that heparin can act via antithrombin III (AT III) directly on the thrombin generated in situ or indirectly on the enzymatic complex, prothrombinase (or on one of its components), responsible for the conversion of prothrombin to thrombin.<sup>1</sup> These two properties, antithrombin activity and antiprothrombinase activity, can be differentiated by mathematical dissection of the thrombin generation curve as previously described.<sup>2</sup> The method consists of determining the course of thrombin generation in clotting plasma, in the presence or absence of the substance to be tested. The  $\alpha_2$ -macroglobulin dependent and independent breakdown constants of endogenous thrombin are independently determined. From the experimental curve and the breakdown constants, the prothrombinase activity can be calculated with the assistance of a computer program. This method was applied to study the mechanism of action of LW 10082, a synthetic sulfated bis-lactobionic acid amide from Luitpold-Werk (Germany).

## RESULTS

The batch of lactobionic acid amide tested dose-dependently inhibits the generation of thrombin in platelet-poor plasma, both in the extrinsic (Fig. 1) and in the intrinsic (Fig. 2) pathways. A prolongation of the lag phase before the apparition of thrombin is seen in the intrinsic pathway. The lactobionic acid also inhibits the

extrinsic pathway thrombin generation in AT III-depleted plasma (Fig. 3) and in AT III and  $\alpha_2$ -macroglobulin depleted plasma (data not shown). However, only a minor effect on thrombin generation was observed in AT III- and heparin cofactor II (HC II)-depleted plasma (Fig. 4) as well as in HC II-depleted plasma (Fig. 5). Thus, HC II is the necessary cofactor for lactobionic acid.

The breakdown constants of endogenous thrombin measured in the presence of different concentrations of lactobionic acid, give a second-order decay constant of  $0.599 \text{ min}^{-1}/\mu\text{g}^{-1}$  of lactobionic acid. Knowing this value, we could, in principle, calculate its influence on prothrombinase activity. However, the fact that LW 10082 acts with HC II as a cofactor introduces a complication in the calculation of prothrombinase. When we compare (Table 1) the concentrations of prothrombin with AT III and HC II in the plasma and in the serum, it becomes clear that we cannot neglect the HC II consumption during the thrombin generation test. This leads to a variation of the breakdown constant of thrombin in time, which can be described as a second-order constant of the form:  $0.599 \times (\text{HC II}) \times (\text{lactobionic acid})$ .

In this way, we observe (Table 2) that lactobionic acid inhibits the prothrombinase activity in both the extrinsic and intrinsic system. Contrary to unfractionated heparin, the lactobionic acid amide inhibits the prothrombinase activity in the extrinsic pathway even at concentrations that cause only a small inhibition of thrombin activity.

## CONCLUSION

The lactobionic acid amide, LW 10182, inhibits thrombin via HC II. From the second-order breakdown constant of endogenous thrombin, we calculate that, on a weight basis, the lactobionic acid LW 10082 has 5% of

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*From the University of Limburg, Department of Biochemistry, Maastricht, The Netherlands and \*Hôpital Purpan, Lab. d'Hémostase, Toulouse France.*

Reprint requests: Dr. Béguin, University of Limburg, Department of Biochemistry P.O. Box 616, 6200 MD Maastricht, The Netherlands.

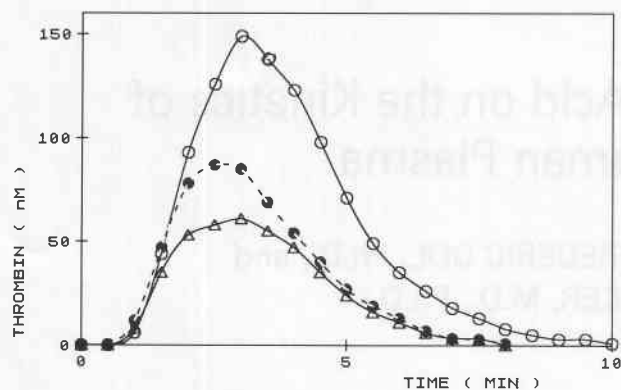


FIG. 1. Influence of lactobionic acid in the extrinsic system. ○: control; ●: 5 µg/ml lactobionic acid; △: 10 µg/ml lactobionic acid.

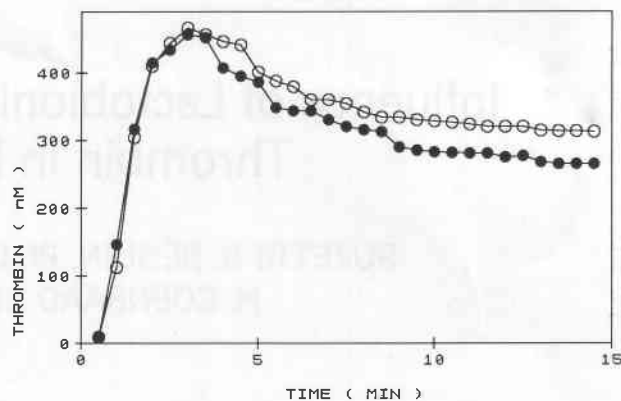


FIG. 4. Influence of lactobionic acid in antithrombin III- and heparin cofactor II-depleted plasma (extrinsic system). ○: control; ●: 10 µg/ml lactobionic acid.

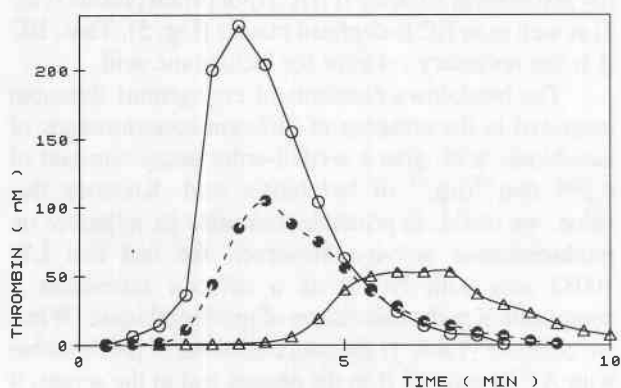


FIG. 2. Influence of lactobionic acid in the intrinsic system. ○: control; ●: 5 µg/ml lactobionic acid; △: 10 µg/ml lactobionic acid.

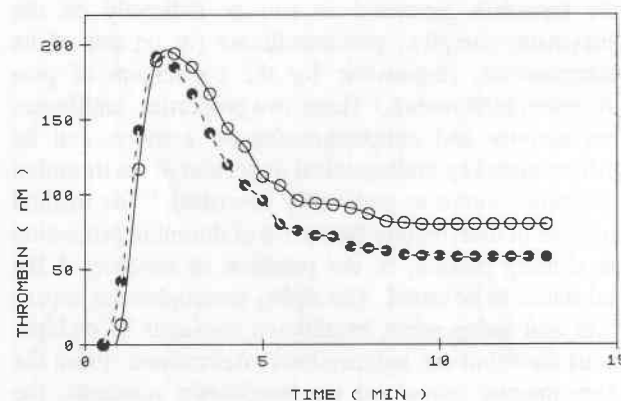


FIG. 5. Influence of lactobionic acid in heparin cofactor II-depleted plasma (extrinsic system). ○: control; ●: 10 µg/ml lactobionic acid.

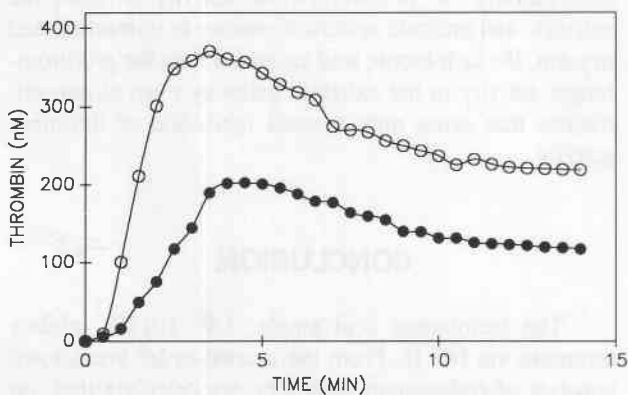


FIG. 3. Influence of lactobionic acid in antithrombin III-depleted plasma (extrinsic system). ○: control, ●: 10 µg/ml lactobionic acid.

TABLE 1. Comparison of Protein Concentrations in Plasma and in Serum

	Antithrombin III (µM)	Heparin Cofactor II (µM)	Prothrombin (µM)
Plasma	3.0	1.0	1.5
Serum	1.5	0	0

TABLE 2. Inhibition of Thrombin and Prothrombinase Activity by Lactobionic Acid in the Extrinsic and Intrinsic Pathways\*

Conc. (µg/ml)	Extrinsic		Intrinsic	
	Ila†	IIase†	Ila	IIase
5	29	14	74	35
10	45	24	88	54

\* The inhibitions are expressed in percent inhibition of a noninhibited control run in parallel.

† Ila: thrombin; IIase: prothrombinase.

the activity of unfractionated heparin (standard heparin  $11.65 \text{ min}^{-1}/\mu\text{g}^{-1}$ ). A direct comparison is not possible, however, because of the difference in cofactor consumption. Both in the extrinsic and the intrinsic system, LW 10082 inhibits the rate of conversion of prothrombin. Moreover, in the intrinsic system one sees an increase of the lag phase before the formation of thrombin. This observation strongly suggests that the lactobionic amide LW 10082 inhibits intrinsic prothrombinase activity by inhibiting the feedback activation of Factor VIII. This hypothesis remains to be confirmed.

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